



Armed Forces College of Medicine AFCM



Cardiopulmonary Module Applied Clinical Chemistry of Pulmonary Diseases

By

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INTENDED LEARNING OBJECTIVES (ILO)



By the end of this lecture the student will be able to:

Interpret the biochemical basis of different clinical disorders related to pulmonary system





Tricarboxylic acid cycle

(TCA)

also called Citric Acid Cycle

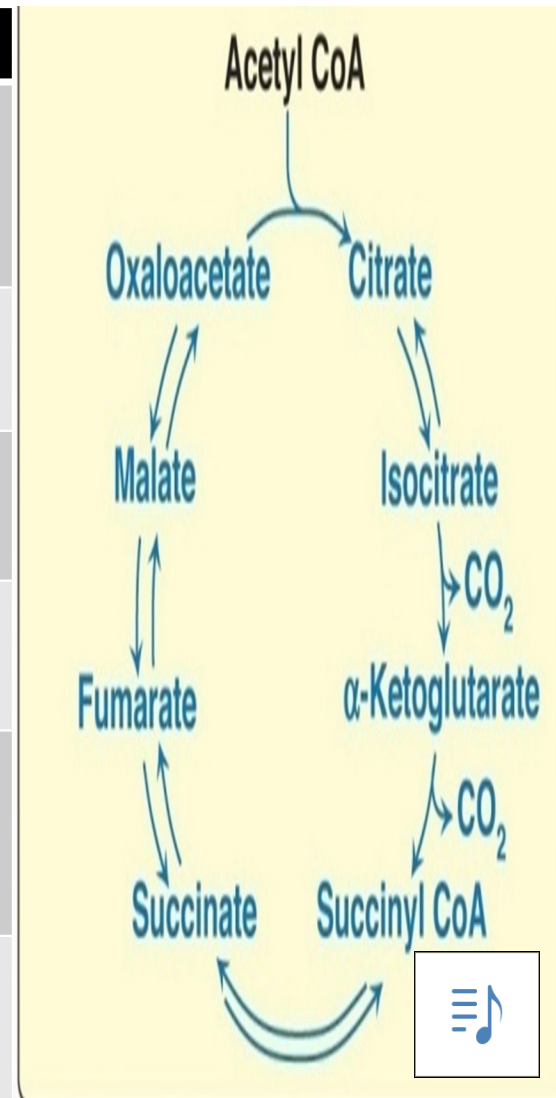
or

Krebs Cycle

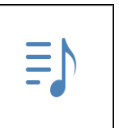


Overview of TCA cycle

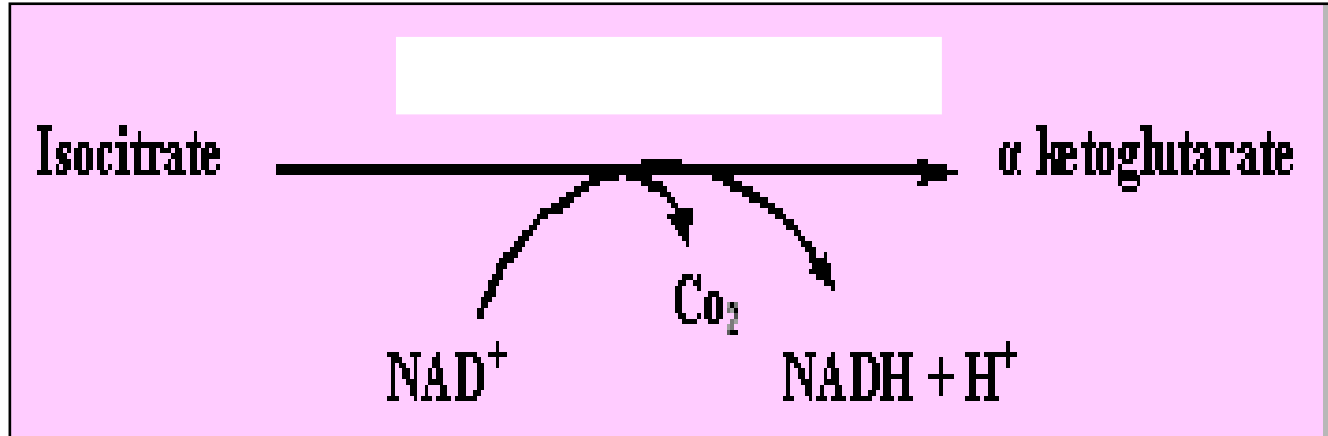
Cellular location in eukaryotes	Mitochondria
Number of reactions	8
Inputs (for complete oxidation of 1 glucose molecule)	2 acetyl CoA, 6 NAD, 2 FAD, 2 ADP
Outputs (for complete oxidation of 1 glucose molecule)	4 CO₂, 2 CoA, 6 NADH, 2 FADH₂, 2 ATP
The initial acceptor	Oxaloacetate that is reformed at the end of the cycle
Key regulatory enzymes	1- Citrate Synthase 2- Isocitrate Dehydrogenase 3- α Ketoglutarate



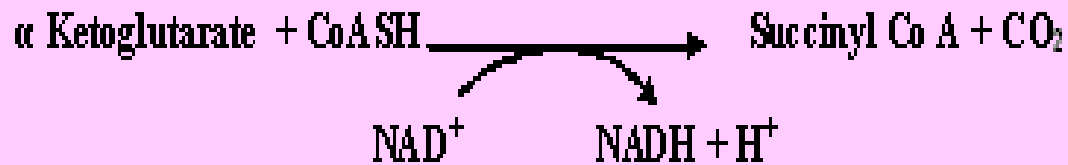
**Illustrate the
deficient parts
of the following
reaction in TCA
cycle**



1-



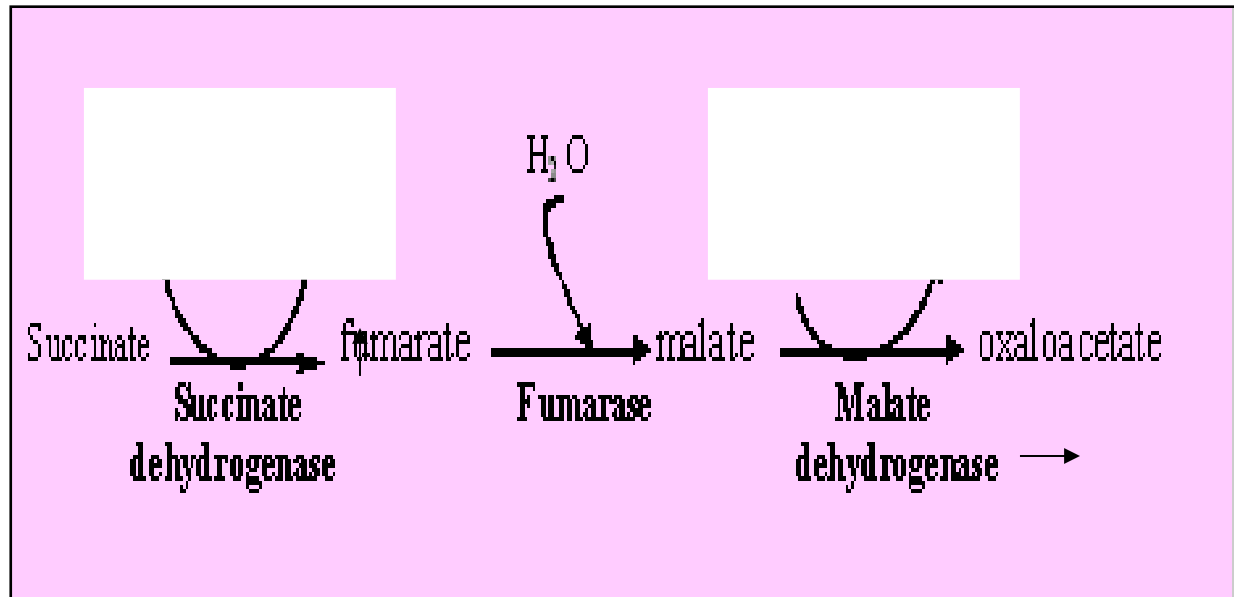
2- Oxidative Decarboxylation of α Ketoglutarate to Succinyl CoA catalyzed by α Ketoglutarate dehydrogenase complex (K



**3
ATP**



5- Oxidation of Malate to Oxaloacetate by **malate dehydrogenase**

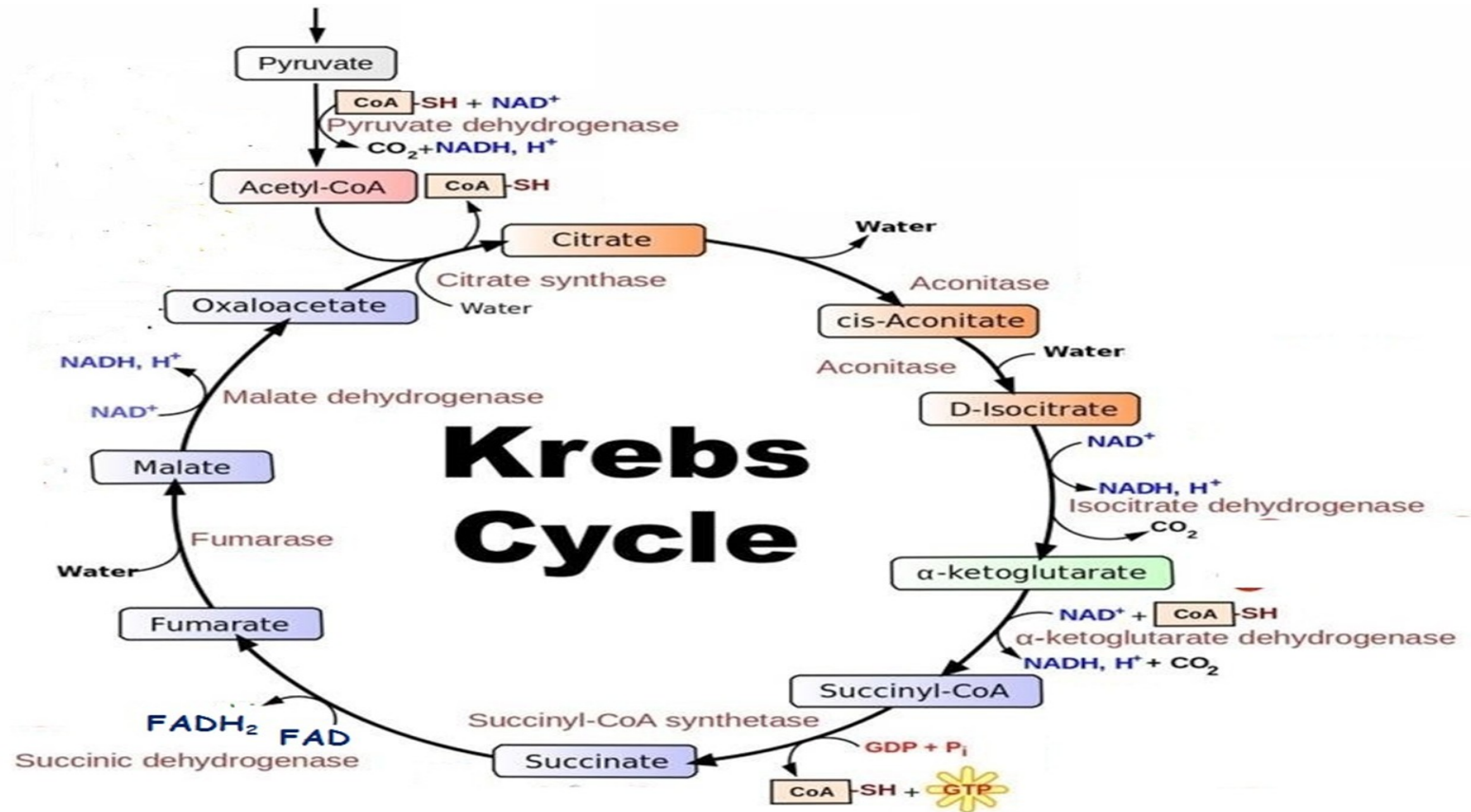


4 members of the vit B-complex play an important role in the TCA cycle (as coenzymes).....**Illustrate them**

- **Thiamin → TPP**
- **Flavin → FAD**
- **Niacin → NAD**
- **Pantothenic Acid → CoASH**



Summarize energy yield of acetyl CoA oxidation via TCA??



TCA = 3 (NADH) + 1 (FADH₂) + 1 ATP



Answer the following question

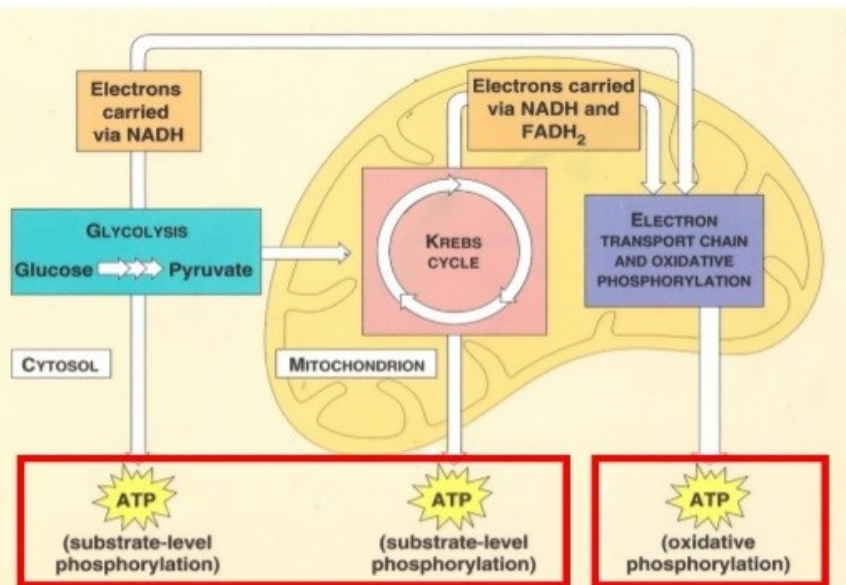
Products of Krebs cycle essential for oxidative phosphorylation are _____

- (a) NADPH and ATP
- (b) Acetyl CoA
- (c) CO₂ and oxaloacetate
- ☒ (d) NADH and FADH₂



Compare between the two methods of Δ ATP synthesis

Substrate level phosphorylation	Oxidative phosphorylation
Direct ATP formation through phosphate transfer from a substrate to ADP	Indirect ATP formation from the oxidation of NADH and FADH ₂ and the pumping of electrons to generate an electrochemical gradient to make ATP
Occurs in glycolysis and krebs cycle	Occurs via the electron transport chain

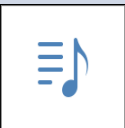


NADH produced in the cytosol cannot pass through the inner mitochondrial membrane, to reach the respiratory chain and be oxidized.

Shuttling of NADH is indirect by two shuttle mechanisms:

1- Glycerol-3P dehydrogenase shuttle

2- Malate-Aspartate shuttle



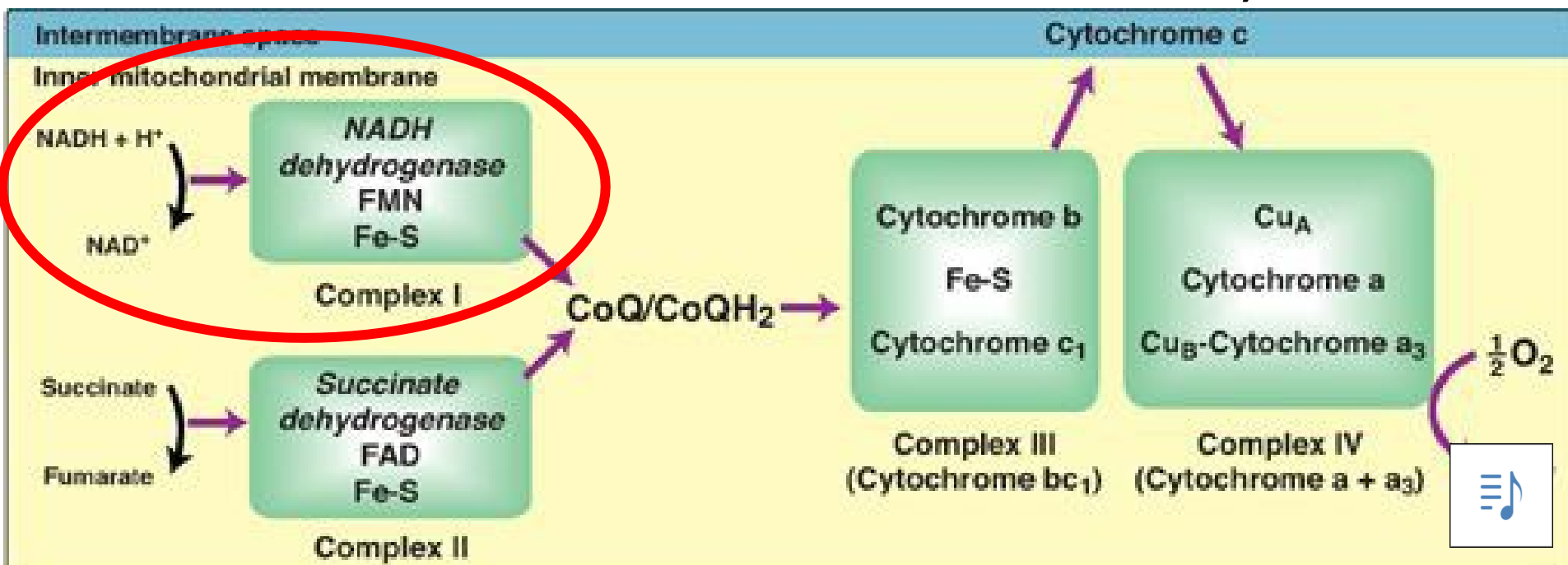
What are the components of the ETC?



Respiratory chain components

1- Complex I [NADH dehydrogenase]:

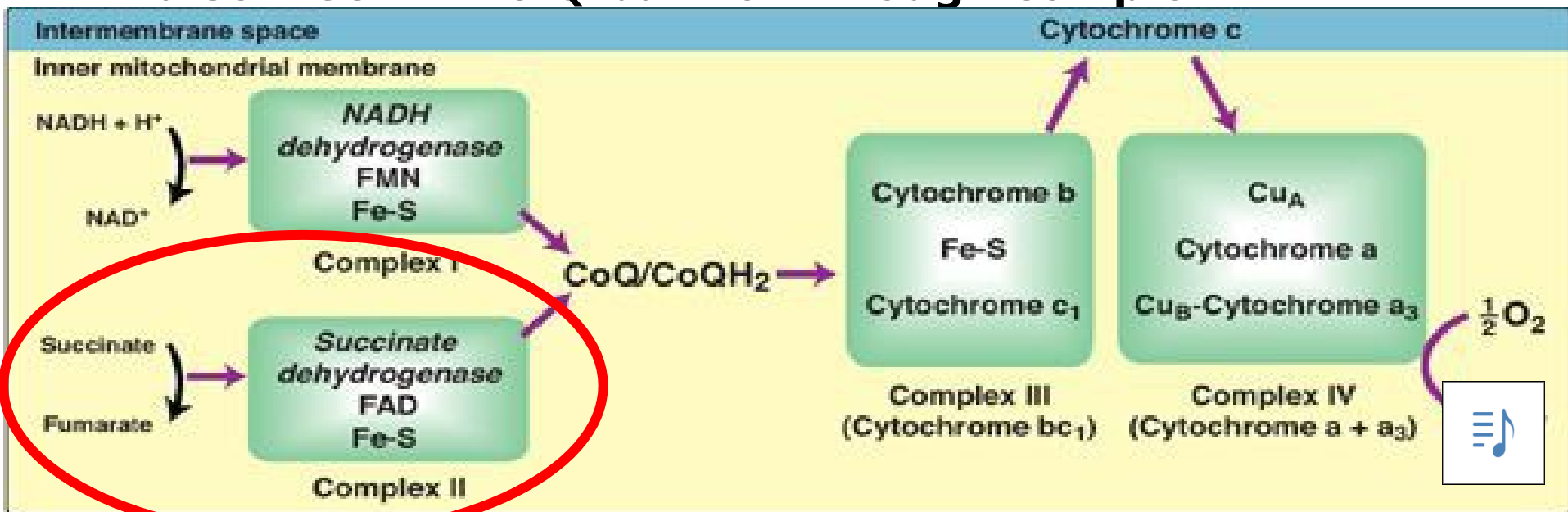
- ❖ It is a flavoprotein containing FMN & FeS as coenzymes.
- ❖ It oxidizes $\text{NADH} + \text{H}^+$ and transfer electrons through FMN and FeS to coenzyme Q which is reduced.
- ❖ During electron transfer, 2 protons (H^+) are pumped across the inner mitochondrial membrane, one ATP is



Respiratory chain components

2- Complex II (Succinate dehydrogenase):

- ❖ It is the site of entry of FADH_2 .
- ❖ **Succinate dehydrogenase is the only membrane bound enzyme of TCA cycle.**
- ❖ It removes 2 H atoms from succinate to coenzyme Q.
- ❖ **No pumping of H^+ occurs at this step.**
- ❖ Glycerol 3-P dehydrogenase and acyl CoA dehydrogenase also free 2 H to Q but not through complex II.

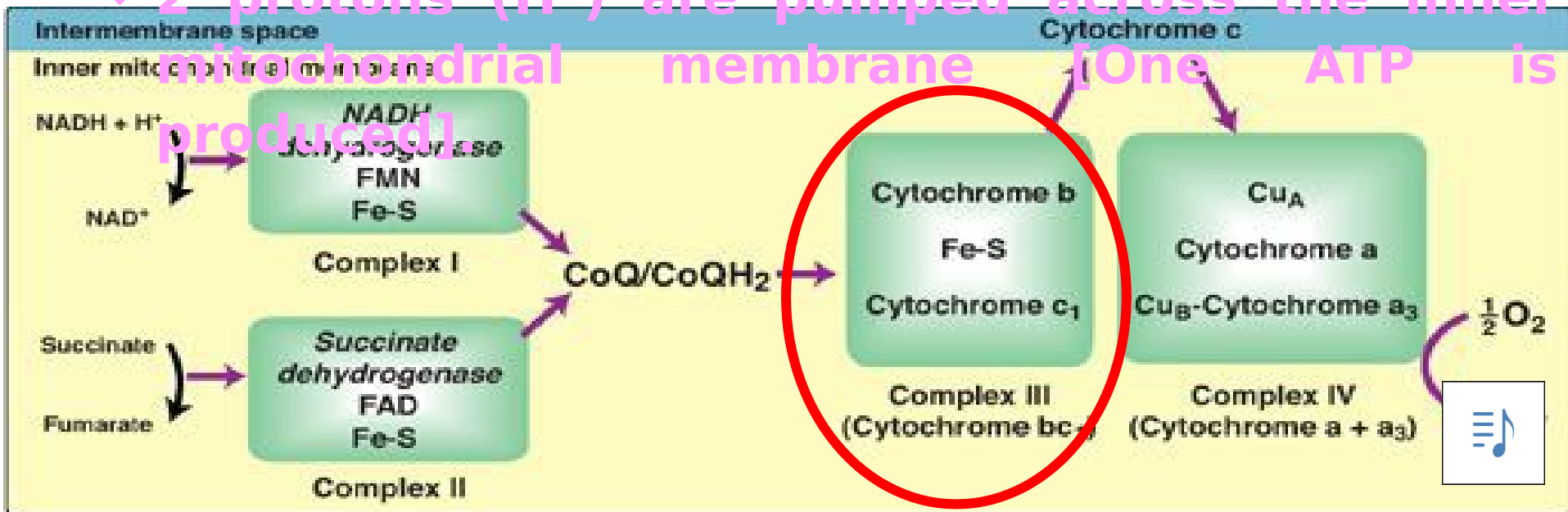


Respiratory chain components

3- Complex III (Cytochrome c reductase):

- ❖ It is composed of *Cytb* and *Cytc₁* and FeS.
- ❖ It catalyzes transfer of electrons from coenzyme Q to *Cytc*.

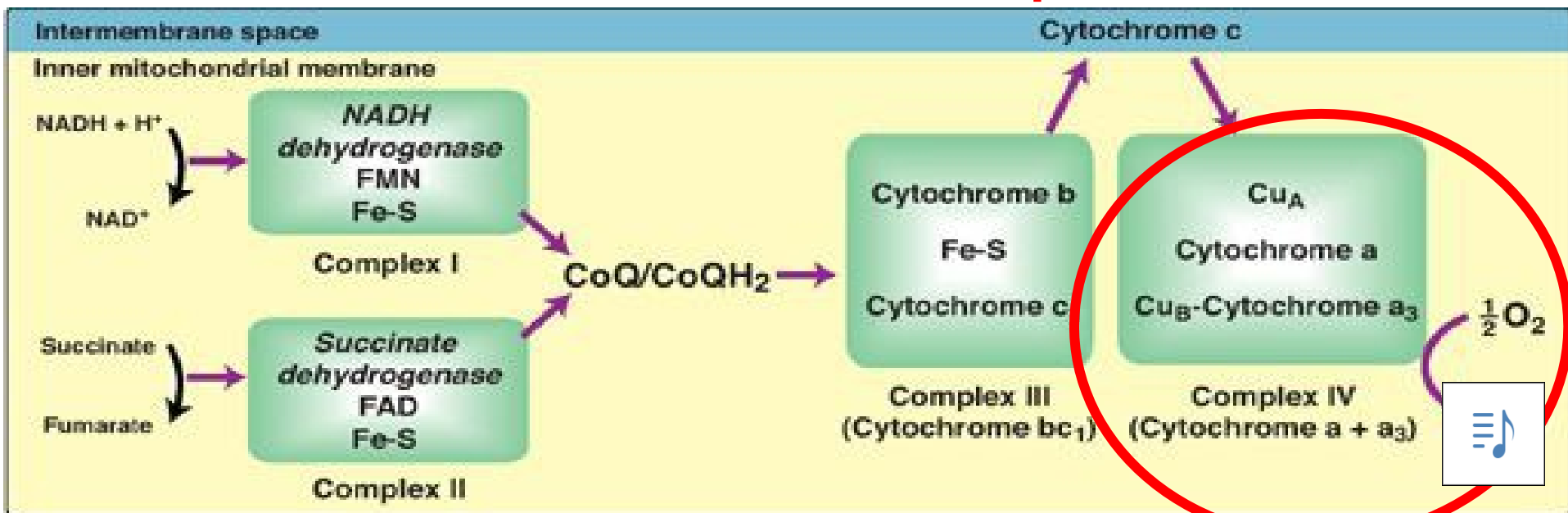
❖ 2 protons (H^+) are pumped across the inner mitochondrial membrane [One ATP is produced].



Respiratory chain components

4-Complex IV (Cytochrome c oxidase):

- ❖ It includes *Cyt a*, *Cyt a3* and 2 copper atoms.
- ❖ Transfers electrons from red. *Cyt c* to O_2 .
- ❖ Iron [$Fe^{+++} \rightarrow Fe^{++}$] and Cu [$Cu^{++} \rightarrow Cu^{+}$] are involved in oxidation/reduction.
- ❖ 2 protons (H^+) are pumped across the inner mitochondrial membrane [One ATP is produced].



What is the mechanism of ATP synthesis through ETC?



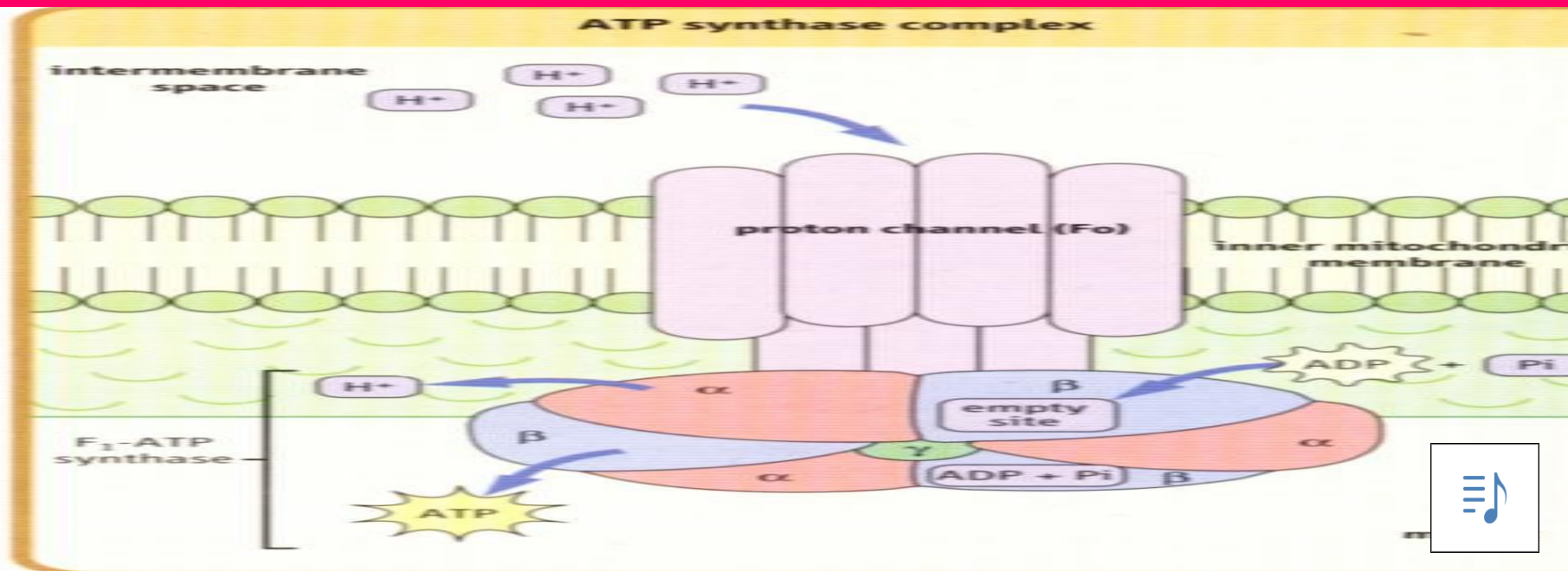
Mechanism of ATP synthesis (**Chemiosmotic theory**)

✓ Transfer of electrons along the ETC → outward pumping of protons across the inner mitochondrial membrane → *a proton and charge gradient (electrochemical gradient)* → Entry of protons through F₀ of ATP synthase → Stimulation → ATP synthesis.



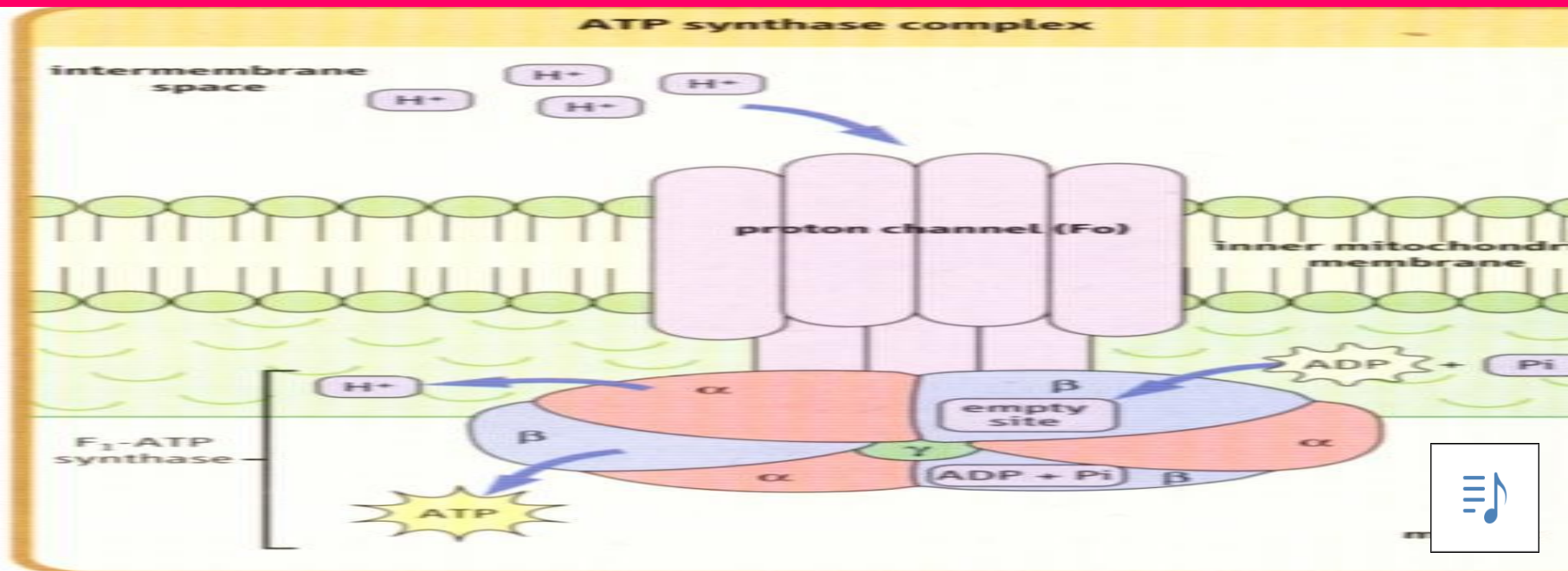
✓ ATP Synthase:

- ✓ Inner Mitochondrial membrane linked enzyme.
- ✓ Consists of several protein subunits, organized into :
 - F₀ sub-complex: discoid transmembrane, forms proton [H⁺] channel.
 - F₁ sub-complex: [3 alternating α and β subunits] which is projected into the matrix and attached to F₀ through rotatory



✓ F1 sub-complex:

- ✓ Has ATP, ADP and Pi binding sites [on β subunit].
- ✓ The flow of H through F₀ → Rotation → Activation of F₁ → Formation of ATP from ADP + Pi.
- ✓ ATP synthase only capture 68% of energy, the rest is released as heat [keeping body temp; driving the oxidation towards completion].

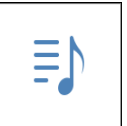


CASE SCENARIO

- Fumes developed at museum and several people started choking and collapsing. The authorities tell you 120 people are already pronounced dead at the scene. You noted a musty bitter almond odor when you enter the gallery.



What is the poison?



Cyanide Poisoning



What is the
cellular
mechanism of
cyanide
poisoning?

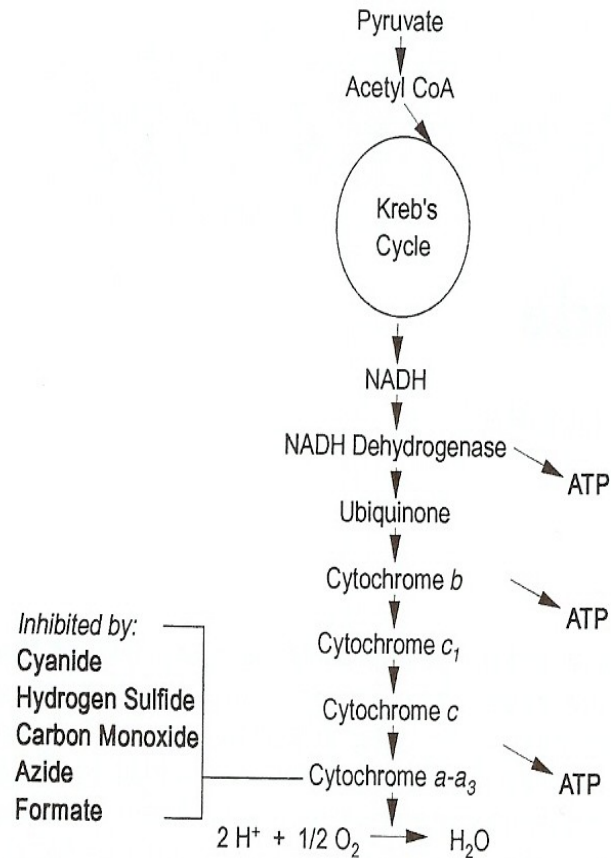


Mechanism of action of Cyanide

- Inhibits cellular respiration
 - Cytochrome a-a3



Kreb's cycle and Cyanide

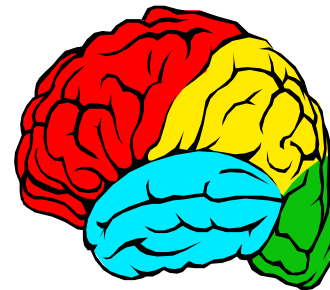


**What are the
clinical effects
of cyanide?**



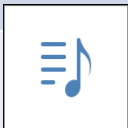
Clinical Effects of Cyanide

- CNS
 - Headache
 - Dizziness
 - Seizures
 - Coma



What are the inhibitors of oxidative phosphorylation?

Comple x	Inhibitor(s)
Comple x 1	Rotenone, an insecticide binds to complex I and prevents the reduction of ubiquinone. Amytal and other barbiturates: prevent electron transfer from iron sulfur centers to ubiquinone.
Comple x 2	Malonate which acts as a competitive inhibitor of succinate.
Comple x 3	Antibiotics inhibit electron transfer through complex III by blocking transfer of electrons from Co Q to iron – sulfur proteins.
Comple x 4	Cyanide and carbon monoxide inhibit Complex IV resulting in impairment of the energy



Answer the following question

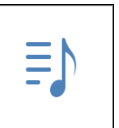
Which of the following has the highest redox potential in the respiratory chain?

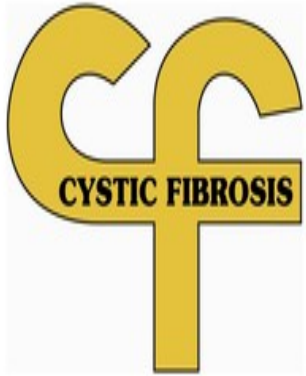
- ☒ (A) O₂
- (B) Ubiquinone
- (C) NAD
- (D) FAD
- (E) FMN



Explain the biochemical basis of:

- 1) Cystic fibrosis
- 2) α 1 antitrypsin deficiency
- 3) Respiratory distress syndrome





What is cystic fibrosis (CF)?

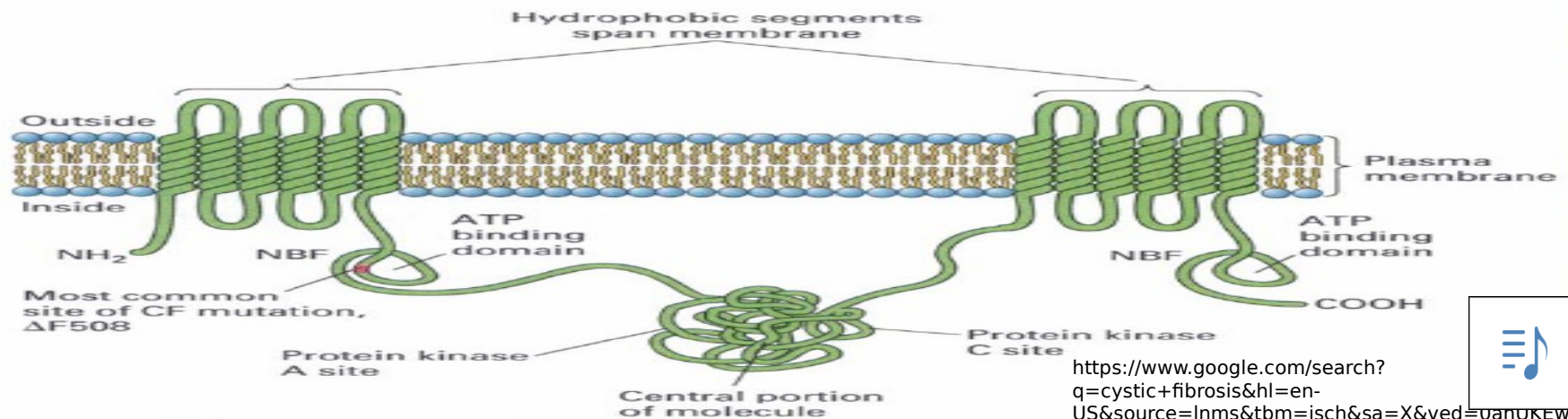
Chronic, progressive and life limiting **autosomal recessive** genetic disease characterized by chronic respiratory disease, pancreatic insufficiency, elevation of sweat electrolytes and male infertility



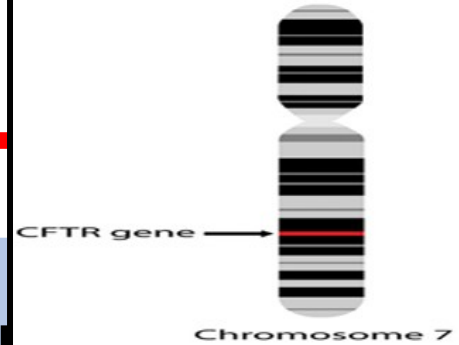
What causes CF?

Defect in the gene that encodes a membrane protein called cystic fibrosis transmembrane conductance regulator, or **CFTR**

CFTR



Genetics of CF (1)



The most common mutation is a 3 base pair deletion leading to absence of phenylalanine at position 508 ($\Delta F508$) of the CFTR protein



The mutant protein is not correctly maturing and reaching the plasma membrane

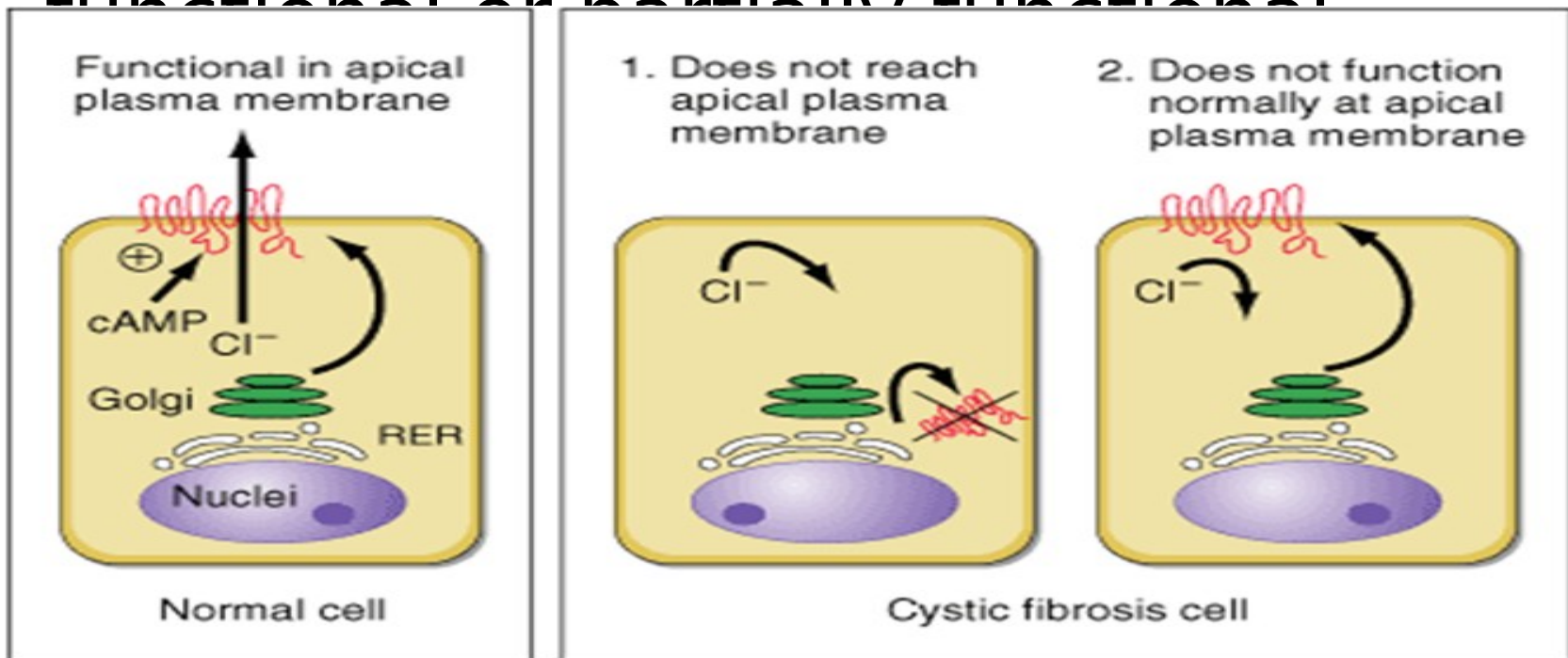


$\Delta F508$ mutation leads to improper processing and intracellular degradation of the CFTR protein



Genetics of CF (2)

Other mutations in the CF gene produce fully processed CFTR proteins that are either non-functional or partially functional



α 1 antitrypsin (AAT)

Most AAT found in plasma is synthesized and secreted by the liver

Extrahepatic synthesis occurs in monocytes and alveolar macrophages (important in the prevention of local tissue injury by elastase)

Is also present in blood and other body fluids

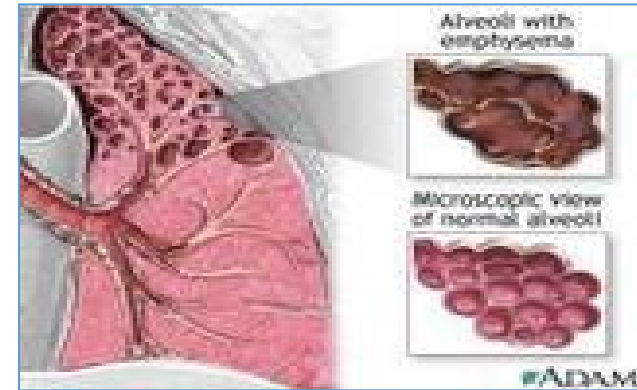


Role of $\alpha 1$ antitrypsin in the lungs

It inhibits proteolytic enzymes including elastase enzyme which is secreted by neutrophils and degrades elastin of alveolar walls as well as other structural proteins in a variety of tissues



α 1 antitrypsin deficiency (1)



https://www.google.com/search?q=emphysema&hl=en-US&source=Inms&tbm=isch&sa=X&ved=0ahUKewjSIMXqh_HjAhXMxoUKHXxABgAQ_AUIECgB&biw=1366&bih=662

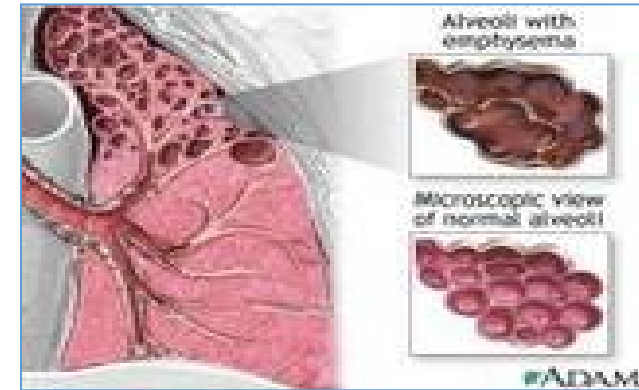
Results from different mutations of AAT gene causing deficiency of the protein

The most clinically widespread is one single purine base mutation (GAG to AAG, resulting in the substitution of lysine for glutamic acid at position 342 of the protein)

The mutation causes the normally monomeric AAT to polymerize within the endoplasmic reticulum of hepatocytes, resulting in decreased secretion of AAT from the liver



α 1 antitrypsin deficiency (2)



https://www.google.com/search?q=emphysema&hl=en-US&source=Inms&tbm=isch&sa=X&ved=0ahUKewjSIMXqh_HjAhXMxoUKHXxABgAQ_AUIECgB&biw=1366&bih=662

Consequently, the polymer that accumulates in the liver may result in cirrhosis and the blood levels of AAT are reduced, decreasing the amount that gets to the alveoli

This causes loss of elastase enzyme inhibition resulting in degradation of elastin of alveolar walls inducing a disease called emphysema



Smoking and Emphysema

Methionine 358 in AAT is required for the binding of the inhibitor to its target proteases.

Smoking causes the oxidation and subsequent inactivation of the methionine, thereby rendering the inhibitor powerless to neutralize elastase.

Smokers with AAT deficiency, therefore, have a considerably elevated rate of lung destruction and a poorer survival rate than nonsmokers with the deficiency.



Respiratory distress syndrome (RDS)

- RDS is a common breathing disorder that affects newborns.
- RDS occurs most often in babies born preterm due to lack of surfactant in the lungs.



What is the laboratory evidence for CFTR dysfunction?

- 1) Two elevated sweat chloride concentrations on two separate days (>70 mEq/L)**
- 2) Identification of two CF mutations**



Answer the following question

You're educating the parents of an 8-month-old child, who was recently diagnosed with cystic fibrosis, about the disease. You explain to the parents that the child has a gene mutation on the _____. The gene that is specifically mutated is called?

- A. endocrine glands; Hbg S gene
- ☒ B. exocrine glands; CFTR gene
- C. endocrine glands; Chromosome 21
- D. exocrine glands; HTT gene



Key points

- The **citric acid cycle** – also known as the TCA cycle (tricarboxylic acid cycle) or the Krebs cycle – is a series of chemical reactions used by all aerobic organisms to release stored energy through the oxidation of acetyl-CoA derived from carbohydrates, fats, and proteins.
- The **electron transport chain** is a series of proteins and organic molecules found in the inner membrane of the mitochondria. Electrons are passed from one member of the transport chain to another in a series of redox reactions. Energy released in these reactions is captured as a proton gradient, which is then used to make ATP in a process called **chemiosmosis**.
- Cystic fibrosis is caused by mutations in the gene encoding the CFTR protein, a Cl⁻ transporter. While Alpha-1 antitrypsin (AAT) deficiency is a genetic disorder that results from different mutations of AAT gene.



References

- "Lippincott's Illustrated Reviews in Biochemistry" by P.C.Champe, R.A.Harvey and D.R.Ferrier
- "Harper's Biochemistry" by R.K.Murray, D.K.Granner, P.A. Mayes and V.W.Rodwell.
- Fundamentals of Clinical Chemistry (Tietz) Sixth
- "Textbook of Biochemistry with Clinical Correlations" by T.M.Devlin
- **www.namrata.co- *Biochemistry for medics***

Thank

you

